formation into its epoxide derivative, (−)-chlordene epoxide. Although several investigations to rationalize bioactivation of some cyclodiene insecticides have been reported on racemic heptachlor and achiral aldrin (Perry, 1960; Brooks and Harrison, 1963; Hamilton, 1971), our research with optically pure form of insecticides unambiguously established that (−)-chlordene epoxide is toxic, per se, while (+)-chlordene is insecticidal after metabolic conversion to the corresponding (−) epoxide.

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Effect of Differential Heat Treatments on the Protein Quality of Casein and Lactalbumin

The effect of autoclaving (121 °C for 30 or 90 min) in the presence and absence of lactose on the protein quality of casein and lactalbumin was examined. Methods of evaluation were amino acid profile (a.a.p.), 1-fluoro-2,4-dinitrobenzene-available lysine, protein efficiency ratio, modified PER, net protein ratio, and net protein utilization. The protein quality of casein was less prone to heat damage than that of lactalbumin. The a.a.p. was the least discriminating method of protein evaluation; all of the four different rat assays detected the heat damage to the proteins. Care must be exercised when choosing a method for protein quality evaluation of milk proteins since some procedures will not demonstrate heat-induced changes.

The detrimental effects of heat and of reducing sugars on the protein quality of lactalbumin and casein have been demonstrated previously (Eldred et al., 1946; Davis et al., 1949; Lea and Hannan, 1950; Schroeder et al., 1951; Osner and Johnson, 1975). However, neither Mabee and Morgan (1951) nor Ford and Shorrock (1971) could detect a significant change in the protein quality of casein after air-oven heating (140 °C) or autoclaving (121 °C). Many of the studies on the effect of heat on these two milk proteins were performed in the 1940's and 1950's. Some of these studies used unrealistic temperatures and times, and the methods of evaluating protein quality changes have been replaced by more reproducible tests.

The object of the present study is to determine chemically and biologically the effect of practical heating times and temperatures on the protein value of lactalbumin and casein. The use of amino acid profiles is being advocated for the prediction of protein quality (Happich et al., 1975). Both Bujard et al. (1967) and Boctor and Harper (1968) state that the acid hydrolysis of the protein into its constituent amino acids is a poor measure of protein quality in a heated protein because there is no distinction between biologically available and unavailable amino acids. Furthermore, protein efficiency ratio (PER) is the biological assay required by regulatory agencies in the United States if claims are made concerning the protein quality of a food. Large discrepancies are reported in the literature for the PER values of the casein control diet (Hegarty, 1975). It seemed imperative, therefore, that the effect of a reducing sugar, lactose, and of realistic heating temperatures and times be determined on casein and lactalbumin by measuring amino acid profiles, chemically determined available lysine, and the comparison of four different biological tests for protein quality.

MATERIALS AND METHODS

Casein (Humko Sheffield, Norwich, NY) or lactalbumin (Sigma Co., St. Louis, MO) was placed in shallow aluminum pans to a depth of 3 cm and autoclaved (American Sterilizer Co., Erie, PA; Model 57-CR) at 121 °C using the automatic fast exhaust and dry cycle. Washed lactalbumin was prepared by adding 10 L of distilled water to 1 kg of lactalbumin. The mixture was stirred for 1 min, and then the powder was allowed to settle at room temperature. Most of the water was siphoned off, and the wash procedure was repeated 15 times. The final two washings were with doubly distilled water. The washed lactalbumin was freeze-dried and then either autoclaved for 30 min (see above) or left untreated. The casein plus lactose mixture was prepared by mixing 950 g of casein and 50 g of β-D-lactose in 2.5 L of doubly distilled water. This mixture was freeze-dried, yielding a casein mixture containing approximately 5% lactose. This amount of lactose is similar to the lactose concentration in commercial grade lactalbumin. The material was then autoclaved for 30 min as described above.

The following biological and chemical tests were performed: protein efficiency ratio (PER) (AOAC, 1965), modified PER (McLaughlan, 1976), net protein utilization (NPU) (Miller and Bender, 1955), net protein ratio (NPR)

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Table I. Effect of Heat and Lactose on PER and Lysine Content of Lactalbumin and Casein

	lactalbumin				
	unheated	A-30[a]	A-90[a]	WL[a]	WL:A-30
PER	3.46 ± 0.04[a]	2.61 ± 0.06[c]	1.17 ± 0.09[e]	3.51 ± 0.03[a]	2.87 ± 0.05[b]
FDNB-Lys[a] (mg/g of protein)	82.30 ± 1.19[a] (8)	70.93 ± 0.35[c] (8)	65.59 ± 0.79[d] (8)	74.06 ± 1.54[b] (5)	69.71 ± 0.99[c] (6)
a.a.p.-Lys[a] (mg/g of protein)	97.1	92.1	88.4	102.8	98.9
	casein				
	unheated	A-30	A-90	LC[a]	LC:A-30
PER	2.85 ± 0.07[b]	2.75 ± 0.06[bc]	2.77 ± 0.03[bc]		2.08 ± 0.09[d]
FDNB-Lys (mg/g of protein)	75.66 ± 0.67[b]	70.63 ± 0.87[c]	68.36 ± 0.73[cd] (6)[b]	75.23 ± 1.19[b] (5)	52.90 ± 1.29[e] (5)
a.a.p.-Lys (mg/g of protein)	78.4		79.3		62.3

[a] Abbreviations: FDNB-Lys, FDNB-available lysine; a.a.p.-Lys, amino acid profile lysine value; A-30, A-90, autoclaved, 121 °C for 30 or 90 min; WL, washed lactalbumin; LC, unheated casein + 5% lactose. [b] Lactalbumin and casein, A-90 values, are significantly different at $p < 0.05$. [c] PER and FDNB lysine values are analyzed by one-way analysis of variance at $p < 0.01$. For both these assays the data for casein and lactalbumin are analyzed together to facilitate comparison between proteins and between heat treatments. Values with the same letter are not significantly different. All values are means ± SEM for the PER values from ten samples (rats); the number of samples for FDNB-lysine is in parentheses.

Table II: Lactose Concentration in Lactalbumin and Casein Samples[a]

sample	% lactose
casein	0
casein + 5% lactose (LC)	4.45
washed lactalbumin (WL)	0
lactalbumin	1.60

[a] All values are the mean of triplicate samples. There was less than 5% variation within replicates.

(Bender and Doell, 1957), amino acid analysis (Spackman et al., 1958); available lysine (Booth, 1971) and lactose (Erickson and Richardson, 1958). All rats used in the biological tests were 21-day old, male Sprague-Dawley (ARS, Madison, WI) with an initial body weight of 45–50 g.

RESULTS AND DISCUSSION

The protein quality of lactalbumin is detrimentally affected by autoclaving (Table I). The PER values decrease significantly when unheated lactalbumin is autoclaved for 30 min (A-30) ($P < 0.01$) or for 90 min (A-90) ($P < 0.001$). The loss of protein quality is reflected in the concurrent decrease ($P < 0.01$) in the concentration of 1-fluoro-2,4-dinitrobenzene (FDNB)-available lysine. Since this lactalbumin sample contains 1.6% lactose (Table II), the loss of bioavailable lysine is probably due to the Maillard reaction. Lactalbumin appears more stable to heat in the absence of lactose, because autoclaved, washed lactalbumin (WL:A-30) has a higher PER value ($P < 0.01$) than its unwashed counterpart A-30 (Table I). The FDNB lysine values are identical for A-30 and WL:A-30, indicating that changes within the protein other than the loss of bioavailable lysine (for example, a possible loss of available methionine) have contributed to the lower PER value of A-30. A reduction in available methionine occurs in roller-dried milk when compared to fresh milk (Pieniazek et al., 1975). Therefore, the protein quality of lactalbumin is damaged by heating, but the damage can be lessened by removing lactose.

Autoclaving has a lesser effect on the protein quality of casein when compared with the results obtained for lactalbumin (Table I). In part this is due to a lack of lactose associated with the protein (Table II). In the presence of lactose, casein (LC:A-30) is detrimentally affected by heat, but not nearly to the extent when lactalbumin (A-30) is compared to their unheated controls (Table I). FDNB lysine values of the casein samples demonstrate the stability of lysine in the absence of lactose (A-30) and its loss in the presence of lactose after autoclaving for 30 min (LC:A-30). The cystine content of the proteins casein and lactalbumin could explain some of their differential response to autoclaving. Casein contains 10% of the cystine found in lactalbumin (Block and Bolling, 1945). This lower concentration could inhibit the formation of protein–protein cross-linkages during heating that would decrease protein quality (Bjarnson and Carpenter, 1970).

There is a discrepancy between the amino acid profile (a.a.p.) lysine values and either the FDNB lysine or PER values in both proteins. The lysine values obtained from the amino acid profile for lactalbumin do not accurately reflect the overall loss of protein quality of the heated samples (Table I). In these two proteins there is, in general, a poor correlation between the a.a.p. lysine and FDNB lysine values, indicating that the a.a.p. lysine test overestimates the amount of bioavailable lysine. The agreement between these two measures of lysine is less after heat

Table III: Protein Quality of Unheated and Heated Casein and Lactalbumin Using Four Different Rat Bioassays[a]

	casein		lactalbumin	
bioassay	unheated	A-90[b]	unheated	A-90
PER	2.85 ± 0.04	2.77 ± 0.03	3.46 ± 0.04	1.17 ± 0.09
r.p.q.[b]	82.36[b]	80.05[b]	100[a]	33.81[c]
Mod.-PER	4.07 ± 0.07	3.95 ± 0.08	4.88 ± 0.09	1.97 ± 0.14
r.p.q.	83.40[b]	80.94[b]	100[a]	40.37[c]
NPR	3.93 ± 0.06	3.87 ± 0.07	4.67 ± 0.09	2.20 ± 0.13
r.p.q.	84.15[b]	82.86[b]	100[a]	47.11[c]
NPU	11.11 ± 0.22	10.86 ± 0.15	13.27 ± 0.55	6.47 ± 0.42
r.p.q.	83.72[b]	81.83[b]	100[a]	48.75[c]

[a] All values are means ± standard error of the mean. All values are the mean of ten rats, except the NPU values which are of six rats. Letters which are different represent significant differences ($p < 0.01$) between treatments within each bioassay. [b] Abbreviations: r.p.q., relative protein quality; A-90, autoclaved, 121 °C for 90 min.

treatments for both lactalbumin and casein.

Four rat bioassays were used to examine the effect of heat on casein and lactalbumin. The absolute values of the four assays are listed for the unheated and 90-min autoclaved samples of lactalbumin and casein (Table III). Also listed are relative protein quality (r.p.q.) values (unheated lactalbumin = 100). Table III demonstrates that casein is significantly ($P < 0.01$) less heat-labile than lactalbumin regardless of the assay method. The extent of protein quality loss in lactalbumin varies depending on the bioassay used. The differing r.p.q. values of lactalbumin:A-90 correlate with values obtained with low-quality proteins by McLaughlan (1976). Yanez and McLaughlan (1970) believe that PER underestimates protein quality of lower quality proteins because it has no measure of maintenance protein needs. The NPR and NPU overestimate it due to protein conservation in the rats fed a nonprotein diet. The modified PER values are likely to be the most accurate measure of protein quality (McLaughlan and Keith, 1975); they usually compare well with the more complex and accurate slope–ratio assay of Hegsted and Chang (1965). This is caused by the realistic estimate of the maintenance protein requirement found within the calculations of the modified PER.

In summary, it has been determined that the protein quality of lactalbumin is more heat-labile than that of casein. These observations are important in deciding if lactalbumin or casein is to be used as the reference protein in studies of protein quality of foods. The degree of destruction of protein quality can be reduced by washing the lactalbumin, which results in the removal of the lactose. The method of evaluation of changes of protein quality should be a bioassay, specifically the modified PER, since the amino acid profile technique does not reflect the loss of bioavailability of some essential amino acids.

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Production of Nonglandular Terpenoid Aldehydes within Diseased Seeds and Cotyledons of *Gossypium hirsutum* L.

Cottonseed and cotyledons of germinating seedlings were examined for the presence of fungal-induced, nonglandular, toxic terpenoids. The terpenoid aldehyde hemigossypol was produced in cottonseed during exposure to 9 days of nearly continuous rainfall in the field. However, no productions of terpenoid aldehydes was observed in mature seeds weathered under normal rainfall or deteriorated by *Aspergillus niger* at constant 20% seed moisture. Hemigossypol and gossypol were produced in isolated lesions and vascular tissues of cotyledons of glandless seedlings inoculated with conidia of *Verticillium dahliae* and *Colletotrichum dematium*. Because terpenoids are formed in deteriorating seeds only under extremely moist conditions conducive to germination or rapid deterioration, they should not be a problem in the use of glandless cottonseed for food.

Gossypol, the major terpenoid aldehyde found in pigment glands of cottonseed (*Gossypium hirsutum* L.), is formed by peroxidative dimerization of the sesquiterpenoid aldehyde hemigossypol (Veech et al., 1976). Only trace amounts of hemigossypol occur in glands of cottonseed (Stipanovic et al., 1975), but this terpenoid aldehyde may be formed in major amounts in cells outside glands in response to fungal infection. Considerable amounts of hemigossypol and lesser amounts of gossypol, for example, form in vascular and boll tissue infected by fungi (Bell and Stipanovic, 1978; Mace et al., 1976). Both terpenoids also are formed in the epidermis of healthy roots (Mace et al.,